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(54) Title: ENZYME CONTAINING PREPARATION AND DETERGENT CONTAINING SUCH PREPARATION

(57) Abstract

The enzyme containing preparation contains at least one enzyme, whereby at least 50 % of the enzymatic activity of at least one enzyme is present in the preparation as enzyme crystals, preferably together with a stabilizing agent for the enzyme(s). The detergent contains this preparation. Both the preparation and the detergent can be a granulate or a slurry. Both the preparation and the detergent exhibit good stability and no discoloration.

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# ENZYME CONTAINING PREPARATION AND DETERGENT CONTAINING SUCH PREPARATION

The invention encompasses an enzyme containing preparation containing at least one enzyme and a detergent containing such preparation. In this specification with claims the term "preparation" means either a granulate or a liquid slurry. The slurry can be either anhydrous or substantially anhydrous, or it can be aqueous. These enzyme containing preparations can be used in different fields, e.g. in the field comprising digestive aids and in the detergent field, but their main use is to be found in the detergent field.

In this specification with claims the term "granulate" is to be understood in its widest sense comprising the entire scope from small particles with a size of the order of magnitude around 10 $\mu$m to tablets with a size of the order of magnitude around 1 cm.

Enzyme containing granulates are widely used in industry, mainly as dust free additives to detergents. Reference can be made to e.g. US 4,106,991, US 4,661,452, DOS 2,060,095, and GB 1,362,365.

One of the big problems in regard to enzyme containing granulates is the storage stability of the enzymes when the enzyme containing granulate is mixed with the other detergent components. Even if several methods for stabilization of the enzymatic activity have been devised, the stability of the enzymatic activity in the enzyme granulate containing detergents is still open to improvement. Another problem in regard to enzyme containing granulates is the color, as most enzyme containing granulates will be discolored, if not coated with e.g. $TiO_2$. A third problem in regard to enzyme containing granulates is the smell thereof. Even after a fairly good purification of the enzyme containing fermentation broth the finished product often exhibits an unagreeable strong smell.

Anhydrous or substantially anhydrous slurries are widely used in industry, mainly as additives to liquid detergents. Aqueous slurries can be used as additives to liquid detergents, in the starch industry and in the food industry. If the slurry is aqueous the water activity or the ionic strenght in the slurry has to be

controlled in such manner that the entire amount or substantially the entire amount of the crystalline enzyme is maintained in the crystalline form.

A big problem in relation to liquid detergents is the stability of enzymes added to these. Several stabilization systems have been used in an attempt to overcome this problem. Within the field of low pH formulations both calcium, formate and borate stabilization has been used and are used. Within the higher pH range some of the inhibition methods are also used. But especially within structured liquids a hydrophobic encapsulation has been used, vide GB 2,186,884A. However, all these stabilization systems are open to improvement. Another big problem in regard to liquid, enzyme containing preparations is the color. Most liquid, enzyme containing preparations are colored due to impurities, usually with a dull, brownish color, and also, the color varies somewhat from batch to batch. This means that the manufacturer of liquid detergents will have to compensate for this color, when he wants to produce for instance a blue liquid detergent, and furthermore, this compensation may vary from batch to batch of the liquid enzyme containing preparation, which obviously is disadvantageous. As with granulates, also with liquid detergents the smell can be a problem.

Also, some preparations contain allergy generating impurities, and thus, purer preparations is a desideratum.

Thus the purpose of the invention is the provision of an enzyme containing preparation, which exhibits an improved stability, and which is colorless or almost colorless and of a high purity, and thus without any smell problems, and a detergent containing such enzyme containing preparation.

The enzyme containing preparation according to the invention which contains at least one enzyme is characterized by the fact that at least 50% of the enzymatic activity of at least one enzyme is present in the preparation as enzyme crystals.

Surprisingly it has been found that the enzyme containing preparation according to the invention, when present in a detergent, possesses an equally good or better enzyme stability than otherwise similar but less pure enzyme containing preparations. It is normally assumed that some of the impurities from

the fermentation broth stabilize the enzyme, and that consequently it would be a disadvantage in regard to enzyme stability to purify the enzyme too much, and this assumption is correct. However, if a pure enzyme can be maintained as crystals in the preparations according to the invention it has been found that the stability is almost equal to or better than the stability of less pure preparations. Also, it has been found that the enzyme containing preparation according to the invention possesses improved color and smell characteristics, when the crystallization of the enzyme is performed without later introduction of impurities.

It is to be understood that the enzyme containing preparations according to the invention can be produced in any conventional manner, if only the conventional enzyme starting material, i.e. enzyme in the form of an amorphous powder, usually with a relatively large amount of impurities, or in the form of an enzyme solution or slurry, is substituted by an enzymatic starting material, in which at least 50% of the enzymatic activity is present as enzyme crystals. If the preparation is a granulate, a non limiting list of such usable granulation methods is the previously cited US 4,106,991, US 4,661,452, DOS 2,060,095, and GB 1,362,365.

A preferred form of enzyme crystals is described in co-pending patent applications Nos. 847/90 and 848/90 (our refs. 3411.010-DK and 3411.020), filed on the same date as this application. Other types of enzyme crystals usable in the enzyme containing preparation are known per se. Reference can be made to DK 872/86, US 4,699,882, and Northrop, J.H. et al, Crystalline Enzymes, 2nd edition, New York, 1948. Also, reference can be made to the current catalogues from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO, USA.

A further advantage in relation to the preparation according to the invention is to be found in the washing process in a washing float with chlorine in the tap water. In such washing floats the chlorine will be consumed in the beginning of the washing cycle, and due to the fact that crystalline enzymes are dissolved slower in the washing float than enzymes usually used, the enzymes in the preparation according to the invention will not be present in dissolved form, before part of the chlorine has been consumed, and thus they will be protected

from inactivation by the chlorine. Documentation for this will be presented later in this specification.

In a preferred embodiment of the preparation according to the invention the preparation contains a stabilizing agent for the enzyme(s). In this manner a further improvement of the enzyme stability is obtained. In case of a granulate PVP (polyvinyl pyrrolidone) can be used; in case of a slurry preservatives against microbial growth, sucrose and $Ca^{++}$ can be used as stabilizing agents.

In a preferred embodiment of the preparation according to the invention the preparation contains a protease and at least one other enzyme, whereby substantially 100% of the proteolytic activity is present as crystals. Only a small amount of the crystalline protease or nothing thereof will be dissolved in the preparation according to the invention, and thus, the stability of the other enzyme or the other enzymes in the preparation according to the invention will be improved in this embodiment. If the protease is Savinase® and the preparation contains the lipase Lipolase® as the other enzyme, and if the preparation is a slurry it has been found that in this embodiment of the preparation (with crystalline Savinase®) the lipase exhibits an excellent stability.

In a preferred embodiment of the preparation according to the invention containing a protease and at least one protease sensitive enzyme substantially 100% of the protease sensitive enzyme or the protease sensitive enzymes are present as crystals. Only a small amount of the protease sensitive enzyme(s) or nothing thereof will be dissolved in the preparation according to the invention, and thus, the stability of the protease sensitive enzyme(s) in the preparation according to the invention will be improved in this embodiment. If the protease is Savinase® and the preparation contains the lipase Lipolase® as the other enzyme, and if the preparation is a granulate it has been found that in this embodiment of the preparation (with crystalline lipase) the lipase exhibits an excellent stability.

In a preferred embodiment of the preparation according to the invention more than 90% of the crystals possess a maximum crystal dimension between 0.1 $\mu$m and 500 $\mu$m, preferably between 1 $\mu$m and 100 $\mu$m. Such crystals

are preferably prepared according to co-pending patent applications Nos. 847/90 and 848/90 (our refs. 3411.010-DK and 3411.020-DK), filed on the same date as this application, but they can also be prepared by means of other crystallization methods.

In a preferred embodiment of the preparation according to the invention the enzyme is a protease, lipase, amylase, cellulase, hemicellulase, pectinase, amidase or oxidase, or protein engineered variants of these. These enzymes are common enzymes used as additives in detergents.

In a preferred embodiment of the preparation according to the invention the enzyme is a protease, and the protease is a Subtilisin type protease. Examples are Savinase®, Esperase®, Alcalase®, Subtilisin NOVO or protein engineered variants of these. These enzymes are preferred proteases in detergents.

In a preferred embodiment of the preparation according to the invention the enzyme is a lipase and the lipase is Lipolase®.

In a preferred embodiment of the preparation according to the invention at least 75%, preferably at least 90% of the enzymatic activity of at least one enzyme is present in the granulate as enzyme crystals, preferably of all enzymes. The stability of such preparations is excellent.

In a preferred embodiment of the preparation according to the invention the preparation is a granulate. This preparation is well suited as an additive to a detergent in granulate form.

In a preferred embodiment of the preparation according to the invention the preparation is a slurry.

In a preferred embodiment of the preparation as a slurry the slurry is anhydrous or substantially anhydrous.

In a preferred embodiment of the preparation as a slurry the slurry is aqueous.

In a preferred embodiment of the preparation according to the invention the preparation is used as a detergent additive. This is the main use of the preparation according to the invention.

Also the invention comprises a detergent, which contains the preparation according to the invention.

In a preferred embodiment of the detergent according to the invention the detergent is solid and contains the granulate according to the invention in a concentration of between 0.001 and 10 mg of enzyme protein/g of detergent, preferably between 0.005 and 5 mg of enzyme protein/g of detergent, most preferably from 0.01 to 1 mg of enzyme protein/g of detergent. Depending upon the enzymatic strength of the granulate according to the invention these concentrations will usually be obtained by addition of between 0.01 and 10% w/w of the granulate to the detergent.

In a preferred embodiment of the detergent according to the invention the detergent is liquid and contains the anhydrous or substantially anhydrous slurry according to the invention in an amount of between 0.001 to 10 mg of enzyme protein per g of detergent, preferably from 0.005 to 5 mg of enzyme protein per g of detergent, most preferably from 0.01 to 1 mg of enzyme protein per g of detergent. Such liquid detergents exhibit a satisfactory stability of the enzyme.

Use of binders in relation to the granulate preparations according to the invention is a necessity, and optionally such binders can be carbohydrate binders, e.g. dextrins or cellulose derivatives, for instance hydroxypropyl cellulose, methyl cellulose or CMC.

## EXAMPLES

### EXAMPLE 1

13.3 kg of a powder composition with the formulation

0.5 kg crystalline SAVINASE® with an activity of 244 KNPU/g
1.5 kg bentonite ASB 350 (ECCI)
2.2 kg fibrous cellulose, ARBOCEL BC200
9.1 kg finely grounded sodium sulphate

is granulated in a Lödige mixer FM 50 with 4.5 kg of a binder solution consisting of 3.0 kg of water, 0.15 kg of polyvinyl pyrrollidone K30 and 1.35 kg of a carbohydrate binder. The granulation is performed in a manner as described in US patent No. 4,106,991, Example 1.

Savinase® is an alkaline *Bacillus* protease prepared as indicated in US patent No. 3,723,250. Also, reference can be made to the product sheet for Savinase®, B345 b-GB, March 1988, obtainable from Novo Nordisk A/S on request.

The KNPU proteolytic activity unit is defined in AF 101.10, which on request can be obtained from Novo Nordisk A/S, Denmark.

The granulate is dried in a fluid bed to a water content below 1%, whereafter a light colored granulate is obtained with particle distribution:

11% > 1180 µm
17% > 1000 µm
25% > 850 µm
40% > 710 µm
56% > 600 µm
77% > 500 µm
86% > 420 µm
93% > 355 µm
2.4% < 300 µm

with an activity of 7.3 KNPU/g and with the Hunter color coordinates (L:a:b)=(79.6: -1.3: 11.3).

The granulate is finally sifted to get a product with the particle range 300 µm to 1000 µm and coated with 7% of PEG 4000 and 12% of a 1:1 mixture of $TiO_2$ and kaolin in a manner as described in US patent No. 4,106,991, Example 22.

## EXAMPLE 2

Preparation of a Lipolase® 100 T granulate containing crystalline Lipolase®

The following components are introduced into a Lödige mixer FM 50:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

2.1 kg of carbohydrate binder

0.63 kg of crystalline Lipolase® (Lipolase® produced according to EP 238023)

12.3 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 3.6 kg of water. During and after the spraying the moist mixture is exposed to a compacting and granulation influence from the multiple set of knives, as descibed in Example 1 of US patent no. 4,106,991. When the granulation is finished, the granulate is dried in a fluid bed, and the part thereof defined as product fraction (in this cae 300-900 µm) is separated for quality testing. The undersize fraction, which is not treated and the oversize fraction, which is crushed, are recirculated.

The color is measured according to the Hunter Lab Scale (AF 78):

| | | | |
|---|---|---|---|
| L | = | 70.1 |
| a | = | 0.4 |
| b | = | 12.0 |

The above-mentioned product fraction is coated according to US 4,106,991, Example 22 for storage stability testing.

EXAMPLE 3

Preparation of a Lipolase® 100 T granulate containing amorphous Lipolase® as a reference for comparison

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

2.1 kg of carbohydrate binder

0.63 kg of amorphous Lipolase®

9.7 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 3.0 kg of water.

Color coordinates:

L = 68.0

a = 0.7

b = 15.0

It appears that the color of the preparation in Example 2 (crystalline Lipolase®) is brighter than the color of the preparation in Example 3 (amorphous Lipolase®).

The product fraction is coated for storage stability testing.

The storage stability of the coated granulates produced according to Examples 2 and 3 is tested using accelerated conditions:

2% of the granulate is added to a European heavy duty detergent and stored in closed jars at 50°C. The activity is measured after 0, 1, 3 and 7 days and calculated in percent.

| | Residual activity (%) | | |
|---|---|---|---|
| | 1 day | 3 days | 7 days |
| Example 2: crystalline Lipolase® | 99 | 85 | 69 |
| Example 3: amorphous Lipolase® | 90 | 82 | 59 |

## EXAMPLE 4

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing amorphous Savinase® and crystalline Lipolase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
0.32 kg of crystalline Lipolase®
1.84 kg of amorphous Savinase®
10.7 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 3.3 kg of water. The product fraction is coated for storage stability testing.

## EXAMPLE 5

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing amorphous Savinase® and amorphous Lipolase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350
3.0 kg of fibrous cellulose, Arbocel BC 200
2.1 kg of carbohydrate binder
1.6 kg of amorphous Lipolase®
1.84 kg of amorphous Savinase®
9.7 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 3.3 kg of water.

The product fraction is coated for storage stability testing.

## EXAMPLE 6

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing crystalline Savinase® and amorphous Lipolase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

2.1 kg of carbohydrate binder

1.6 kg of amorphous Lipolase®

11.0 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 0.7 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90 suspended in 2.9 kg of water.

The product fraction is coated for storage stability testing.

## EXAMPLE 7

Preparation of a Savinase®/Lipolase® 3.0/50 T cogranulate containing crystalline Savinase® and crystalline Lipolase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

2.1 kg of carbohydrate binder

0.32 kg of crystalline Lipolase®

12.2 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 0.7 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90 suspended in 3.0 kg of water.

The product fraction is coated for storage stability testing.

The storage stability of the coated granulates produced according to Examples 4 through 7, determined in regard to the protease sensitive enzyme Lipolase®, is tested under accelerated conditions:

4% of the granulate is added to European heavy duty detergent and stored in closed jars at 50°C. The activity is measured after 0, 1, 3 and 7 days and calculated in percent.

| | | Residual activity of Lipolase (%) | | |
|---|---|---|---|---|
| | | 1 day | 3 days | 7 days |
| Example 4: | amorphous Savinase®/- crystalline Lipolase® | 99 | 84 | 77 |
| Example 5: | amorphous Savinase®/- amorphous Lipolase® | 89 | 61 | 39 |
| Example 6: | crystalline Savinase®/- amorphous Lipolase® | 81 | 54 | 45 |
| Example 7: | crystalline Savinase®/- crystalline Lipolase® | 95 | 84 | 77 |

In another example the granulates produced according to Examples 4 through 7, determined in regard to the protease sensitive enzyme Lipolase®, is tested under other accelerated conditions:

4% of the granulate is added to European heavy duty detergent and stored in closed jars at 37°C and 70% relative humidity. The activity is measured after 0, 3, 7 and 14 days and calculated in percent.

| | | Residual activity of Lipolase (%) | | |
|---|---|---|---|---|
| | | 3 days | 7 days | 14 days |
| Example 4: | amorphous Savinase®/- crystalline Lipolase® | 87 | 66 | 41 |
| Example 5: | amorphous Savinase®/- amorphous Lipolase® | 51 | 15 | 5 |
| Example 6: | crystalline Savinase®/- amorphous Lipolase® | 46 | 15 | 4 |
| Example 7: | crystalline Savinase®/- crystalline Lipolase® | 81 | 58 | 36 |

It appears that the best results are obtained with crystalline lipase.

## EXAMPLE 8

Preparation of a Savinase® 4.0 T granulate containing crystalline Savinase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

2.4 kg of carbohydrate binder

11.7 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 1.2 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90, suspended in 2.2 kg of water, 0.11 kg of $Na_2SO_4$ and 0.2 kg of PVP K30.

Color coordinates:

| | | | |
|---|---|---|---|
| | L | = | 79.9 |
| | a | = | 0.8 |
| | b | = | 7.8 |

## EXAMPLE 9

Preparation of a Savinase® 4.0 T granulate containing amorphous Savinase®

Produced according to Example 2 with the following formulation:

0.8 kg of kaolin, Speswhite

3.0 kg of fibrous cellulose, Arbocel BC 200

3.0 kg of amorphous Savinase® concentrate

11.2 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 1.8 kg of carbohydrate binder, 0.2 kg of PVP K30 in 3.0 kg of water.

Color coordinates:

| | | | |
|---|---|---|---|
| | L | = | 48.2 |
| | a | = | 6.0 |
| | b | = | 17.7 |

It clearly appears that the color of the preparation in Example 8 (crystalline Savinase®) is much brighter than the color of the preparation in Example 9 (amorphous Savinase®), to the point, that no $TiO_2$ is needed in the preparation of Example 8.

EXAMPLE 10

Preparation of a Durazym® 6.0 T granulate containing crystalline Durazym®

Produced according to Example 2 with the following formulation:

0.7 kg of bentonite ASB 350

1.0 kg of fibrous cellulose, Arbocel BC 200

0.4 kg of carbohydrate binder

4.3 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 0.354 kg of crystalline Durazym® filter cake produced according to DK patent application no. 847/90, suspended in 1.5 kg of water, 0.07 kg of PVP K30 and 0.4 kg of carbohydrate binder.

Color coordinates:

L = 82.5

a = 1.0

b = 10.0

EXAMPLE 11

Preparation of a Durazym® 6.0 T granulate containing amorphous Durazym®

Produced according to Example 2 with the following formulation:

1.5 kg of bentonite ASB 350

3.25 kg of fibrous cellulose, Arbocel BC 200

0.9 kg of carbohydrate binder

7.7 kg of ground $Na_2SO_4$

2.0 kg of amorphous Durazym®

The mixed dry components are sprayed with 2.5 kg of water, 0.15 kg of PVP K30 and 0.5 kg of carbohydrate binder.

Color coordinates:

L = 52.8

a = 5.2

b = 17.4

It clearly appears that the color of the preparation in Example 10 (crystalline Durazym®) is much brighter than the color of the preparation in Example 11 (amorphous Durazym®), to the point, that no $TiO_2$ is needed in the preparation of Example 10.

## EXAMPLE 12

Preparation of a Savinase® 4.0 T granulate containing crystalline Savinase®

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Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

1.2 kg of carbohydrate binder

11.9 kg of ground $Na_2SO_4$

0.46 kg of dry crystalline Savinase®

The mixed dry components are sprayed with 2.3 kg of water containing 0.2 kg of PVP K30 and 1.2 kg of carbohydrate binder.

## EXAMPLE 13

Preparation of a Savinase® 4.0 T granulate containing crystalline Savinase®

Produced according to Example 2 with the following formulation:

2.0 kg of bentonite ASB 350

3.0 kg of fibrous cellulose, Arbocel BC 200

2.4 kg of carbohydrate binder

11.6 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 1.5 kg of crystalline Savinase® filter cake produced according to DK patent application no. 847/90, suspended in 2.5 kg of water with 0.1 kg of $Na_2SO_4$ and 0.2 kg of PVP K30.

## EXAMPLE 14

Preparation of a Savinase® 6.0 T granulate containing amorphous Savinase®

Produced according to Example 2 with the following formulation:

0.8 kg of kaolin, Speswhite

1.8 kg of fibrous cellulose, Arbocel BC 200

2.8 kg of amorphous Savinase®

12.6 kg of ground $Na_2SO_4$

The mixed dry components are sprayed with 1.8 kg of carbohydrate binder, 0.2 kg of PVP K30 in 3.6 kg of water.

The storage stability of the coated granulates produced according to Examples 12 through 14, determined in regard to Savinase®, is tested under accelerated conditions:

1% of the granulate is added to a European heavy duty detergent and stored in closed jars at 50°C. The activity is measured after 0, 1, 3 and 7 days and calculated in percent.

| | Residual activity of Savinase (%) | | |
|---|---|---|---|
| | 1 day | 3 days | 7 days |
| Example 12: dry crystalline Savinase® | 60 | 58 | 52 |
| Example 13: crystalline Savinase® | 68 | 57 | 49 |
| Example 14: amorphous Savinase® | 60 | 51 | 42 |

It appears that the stability of crystalline Savinase® is better than of amorphous Savinase®.

## EXAMPLE 15

This example illustrates the further advantage in regard to a chlorine containing washing float. The release velocity in seconds, i.e. the time interval needed to dissolve 50, 90 and 95% of the enzyme, appears from the below indicated table.

| | Release velocity (s) | | |
|---|---|---|---|
| | 50% | 90% | 95% |
| Example 12: dry crystalline Savinase® | 57 | 85 | 92 |
| Example 13: crystalline Savinase® | 69 | 116 | 122 |
| Example 14: amorphous Savinase® | 43 | 62 | 59 |

It clearly appears that the crystalline Savinase® dissolves more slowly than the amorphous Savinase®.

EXAMPLE 16

The liquid preparation Lipolase® 100L in an amount of 1%, crystalline Savinase in an amount of 0.2% and dissolved Savinase® in an amount of 0.625% was added to NOVO standard liquid detergent with builder with the following composition:

| | |
|---|---|
| Berol 160 | 15% |
| NANSA 1169/P | 33.3% |
| Coconut fatty acid | 9% |
| Oleic acid | 1% |
| Triethanolamin | 9% |
| Glycerol | 12% |
| Ethanol | 1.5% |
| Trisodium citrate $2H_2O$ | 8% |
| $CaCl_2$ $2H_2O$ | 0.1% |
| NaOH | 1% |
| Water | 10.1% |
| | |
| pH | 8.5 |

The below indicated table shows the residual activity of the dissolved Lipolase® in the liquid detergent after 3 days at 4°C and 35°C.

| | Residual activity after 3 days | |
|---|---|---|
| Sample | 4°C | 35°C |
| Lipolase® + crystalline Savinase® | 66% | 23% |
| Lipolase® + liquid Savinase® | 16% | 0.9% |

It clearly appears that the stability of Lipolase® was better in the presence of crystalline Savinase® than in the presence of the dissolved Savinase®.

EXAMPLE 17

This example illustrates the storage stability of aqueous and anhydrous Savinase® slurries.

The aqueous slurry base was 54% $(NH_4)_2SO_4$ in deionized water.

The anhydrous slurry base was 305 g of Surfactant T9, 140 g $Na_2SO_4$ and 15 g Aerosil 200 which were well mixed and homogenized with an Ultra-Turrax mixer.

8.7 g crystalline Savinase® or 17 g of amorphous Savinase® were mixed with each base. The slurries were homogenized with an Ultra-Turrax mixer.

The slurries were incubated at 60°C for 5 days and the residual activities were measured.

Storage stability

| | Crystalline | | Amorphous | |
|---|---|---|---|---|
| | Aqueous KNPU/g (%) | Anhydrous KNPU/g (%) | Aqueous KNPU/g (%) | Anhydrous KNPU/g (%) |
| 0 days | 63.0(100) | 15.8(100) | 9.18(100) | 7.63(100) |
| 1 - | 65.6(104) | 17.5(111) | 7.45( 81) | 7.05( 92) |
| 5 - | 57.7( 92) | 14.7( 93) | 7.61( 83) | 6.48( 85) |

It clearly appears from the above table that the stability of the crystalline preparations is better than the stability of the amorphous preparations.

## CLAIMS

1. Enzyme containing preparation containing at least one enzyme, wherein at least 50% of the enzymatic activity of at least one enzyme is present in the preparation as enzyme crystals.

2. Preparation according to Claim 1, wherein the preparation contains a stabilizing agent for the enzyme(s).

3. Preparation according to Claim 1 or 2, containing a protease and at least one other enzyme, wherein substantially 100% of the proteolytic activity is present as crystals.

4. Preparation according to Claim 1 or 2, containing a protease and at least one protease sensitive enzyme, wherein substantially 100% of the protease sensitive enzyme or the protease sensitive enzymes are present as crystals.

5. Preparation according to Claims 1 - 4, wherein more than 90% of the crystals possess a maximum crystal dimension between 0.01 $\mu$m and 500 $\mu$m, preferably between 0.1 $\mu$m and 100 $\mu$m.

6. Preparation according to Claims 1 - 5, characterized by the fact, that the enzyme is a protease, lipase, amylase, cellulase, hemicellulase, pectinase, amidase or oxidase, or protein engineered variants of these.

7. Preparation according to Claim 6, characterized by the fact that the enzyme is a protease, and that the protease is a Subtilisin type protease.

8. Preparation according to Claim 6, characterized by the fact that the enzyme is a lipase and that the lipase is Lipolase®.

9. Preparation according to Claims 1 - 8, wherein at least 75%, preferably at least 90% of the enzymatic activity of at least one enzyme is present in the preparation as enzyme crystals, preferably of all enzymes.

10. Preparation according to Claims 1 - 9, wherein the preparation is a granulate.

11. Preparation according to Claims 1 - 9, wherein the preparation is a slurry.

12. Preparation according to Claim 11, wherein the slurry is anhydrous or substantially anhydrous.

13. Preparation according to Claim 11, wherein the slurry is aqueous.

14. Preparation according to Claims 1 - 13, wherein the preparation is used as a detergent additive.

15. Detergent, characterized by the fact that it contains the preparation according to Claims 1 - 14.

16. Detergent according to Claim 15, characterized by the fact that it is solid and contains the enzyme containing granulate according to Claim 10 in a concentration of between 0.001 and 10 mg of enzyme protein/g of detergent, preferably between 0.005 and 5 mg of enzyme protein/g of detergent, most preferably from 0.01 to 1 mg of enzyme protein/g of detergent.

17. Detergent according to Claim 15 characterized by the fact that it is liquid and contains the enzyme containing slurry according to Claim 11 in an amount of between 0.001 and 10 mg of enzyme protein per g of detergent, preferably from 0.005 to 5 mg of enzyme protein per g of detergent, most preferably from 0.01 to 1 mg of enzyme protein per g of detergent.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 90/00340

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 N 9/00, C 11 D 3/386, 7/42

**II. FIELDS SEARCHED**

**Minimum Documentation Searched**[7]

| Classification System | Classification Symbols |
|---|---|
| IPC5 | C 12 N; C 11 D |

**Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched**[8]

SE,DK,FI,NO classes as above

**III. DOCUMENTS CONSIDERED TO BE RELEVANT**[9]

| Category * | Citation of Document,[11] with indication, where appropriate, of the relevant passages[12] | Relevant to Claim No.[13] |
|---|---|---|
| P,X | EP, A2, 0375102 (THE CLOROX COMPANY) 27 June 1990, see claim 1 -- | 1-17 |
| X | WO, A1, 8908703 (GENENCOR, INC.) 21 September 1989, see the whole document -- | 1-17 |
| X | DE, A, 1959603 (ELI LILLY AND COMPANY) 19 November 1970, see the whole document -- | 1-15 |
| X | US, A, 4699882 (KALEVI J. VISURI) 13 October 1987, see abstract -- | 1-15 |

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 3rd April 1991 | 1991 -04- 05 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| SWEDISH PATENT OFFICE | Jack Hedlund |

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/DK 90/00340

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 91-02-28
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| EP-A2- 0375102 | 90-06-27 | JP-A- | 2225599 | 90-09-07 |
| WO-A1- 8908703 | 89-09-21 | EP-A- | 0404817 | 91-01-02 |
| DE-A- 1959603 | 70-11-19 | AT-A- | 295452 | 71-12-15 |
| | | BE-A- | 742215 | 70-05-26 |
| | | FR-A-B- | 2024571 | 70-08-28 |
| | | GB-A- | 1242976 | 71-08-18 |
| | | NL-A- | 6917822 | 70-06-02 |
| US-A- 4699882 | 87-10-13 | AU-B- | 585866 | 89-06-29 |
| | | AU-D- | 4395985 | 86-01-02 |
| | | CA-A- | 1263093 | 89-11-21 |
| | | DK-A- | 8502857 | 85-12-26 |
| | | EP-A- | 0166427 | 86-01-02 |
| | | JP-A- | 61015686 | 86-01-23 |
| | | SU-A- | 1575946 | 90-06-30 |
| | | WO-A- | 86/00336 | 86-01-16 |